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Bilateral caudate and putamen grafts of embryonic mesencephalic tissue treated with lazaroids in Parkinson's disease

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Summary

Five parkinsonian patients were transplanted bilaterally into the putamen and caudate nucleus with human embryonic mesencephalic tissue from between seven and nine donors. To increase graft survival, the lipid peroxidation inhibitor tirilazad mesylate was administered to the tissue before implantation and intravenously to the patients for 3 days thereafter. During the second postoperative year, the mean daily L-dopa dose was reduced by 54% and the UPDRS (Unified Parkinson's Disease Rating Scale) motor score in 'off' phase was reduced by a mean of 40%. At 10–23 months after grafting, PET showed a mean 61% increase of 6-L-[^{18}F]fluorodopa uptake in the putamen, and 24% increase in the caudate nucleus, compared with preoperative values. No obvious differences in the pattern of motor recovery were observed between these and other previously studied cases with putamen grafts alone. The amount of mesencephalic tissue implanted in each putamen and caudate nucleus was 42 and 50% lower, respectively, compared with previously transplanted patients from our centre. Despite this reduction in grafted tissue, the magnitudes of symptomatic relief and graft survival were very similar. These findings suggest that tirilazad mesylate may improve survival of grafted dopamine neurons in patients, which is in agreement with observations in experimental animals.

Keywords: Parkinson's disease; neural transplantation; lazaroid; dopamine; positron emission tomography

Abbreviations: CAPIT = Core Assessment Program for Intracerebral Transplantations; [^{18}F]fluorodopa = 6-L-[^{18}F]fluorodopa; GDNF = glial cell line-derived neurotrophic factor; HBSS = Hank's balanced salt solution; K_i = fluorodopa uptake rate constant; UPDRS = Unified Parkinson's Disease Rating Scale

Introduction

Intracerebral grafts of human embryonic dopamine neurons can reinnervate the striatum and induce clinically useful symptomatic relief in parkinsonian patients (Lindvall *et al.*, 1990, 1992, 1994; Sawle *et al.*, 1992; Widner *et al.*, 1992; Pechanski *et al.*, 1994; Freeman *et al.*, 1995; Kordower *et al.*, 1995, 1996, 1998; Remy *et al.*, 1995; Defer *et al.*, 1996; Wenning *et al.*, 1997; Hagell *et al.*, 1999; Hauser *et al.*, 1999). Although these findings support the idea that a cell replacement therapy might be developed in Parkinson's disease, the clinical trials performed so far have identified major scientific problems that need to be solved before this approach can be used in a large number of patients (Lindvall,

1999). The main obstacle is that relatively large amounts of human embryonic mesencephalic tissue need to be grafted into each patient for therapeutic effects to develop. This is partly because survival of embryonic dopamine neurons is only 3–20% after grafting with currently available procedures. At present, it is estimated that mesencephalic tissue from at least 3–4 human embryos (giving rise to ~100 000–150 000 surviving grafted dopamine neurons) needs to be implanted per side in a patient in order to induce a significant improvement. A similarly low yield of surviving dopamine neurons after transplantation has also been observed in animal experiments (for a review, see Brundin *et al.*, 2000). Several approaches have been found useful to increase dopamine neuron survival after grafting in animals. Exposure of the graft to neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) (Rosenblad *et al.*, 1996; Sinclair *et al.*, 1996; Sautter *et al.*, 1998; Sullivan *et al.*, 1998; Yurek *et al.*, 1998; Wilby *et al.*, 1999) or basic fibroblast growth factor (Mayer *et al.*, 1993; Takayama *et al.*, 1995; Zeng *et al.*, 1996) increases the survival of dopamine neurons at least 2-fold. Lazaroids, i.e. lipid peroxidation inhibitors, counteract the detrimental effects of oxidative stress and have been shown to increase dopamine neuron survival about two-fold in mesencephalic cultures *in vitro* (Frodl *et al.*, 1994; Othberg *et al.*, 1997) and in nigral transplants implanted *in vivo* in rats (Nakao *et al.*, 1994; Grasbon-Frodl *et al.*, 1996; Björklund *et al.*, 1997; Karlsson *et al.*, 1999; Hansson *et al.*, 2000). Finally, caspase inhibitors, which block apoptosis, can support up to a three-fold increase of the number of surviving grafted dopamine neurons in rats (Schierle *et al.*, 1999; Hansson *et al.*, 2000). So far, none of these strategies has been applied in clinical transplantation trials.

Another major obstacle to a widespread application of neural grafting in Parkinson's disease is that the symptomatic relief is incomplete and varies markedly between patients. Clinically valuable improvement has been observed in a majority of operated patients with documented graft survival, and in the most successful cases, it has been possible to withdraw L-dopa treatment (Lindvall *et al.*, 1990, 1992, 1994; Widner *et al.*, 1992; Pechanski *et al.*, 1994; Freeman *et al.*, 1995; Remy *et al.*, 1995; Defer *et al.*, 1996; Wenning *et al.*, 1997; Hauser *et al.*, 1999; Piccini *et al.*, 1999; Hagell *et al.*, 1999, 2000). After intrastriatal grafting, some symptoms, e.g. hypokinesia and on–off fluctuations, improve more markedly than others, such as dyskinesias, postural instability, and impairment of swallowing, speech and gait (Defer *et al.*, 1996; Wenning *et al.*, 1997; Hagell *et al.*, 1999). Incomplete symptomatic relief after intrastriatal implantation of nigral tissue has also been observed in experimental animals with dopamine-denervating lesions (Björklund *et al.*, 1994). Both in animals and man, it has been suggested that absolute recovery would require more extensive reinnervation of denervated striatal and extrastriatal areas. For example, data from experimental models suggest that complete functional recovery may require grafts in the ventral striatum (Lindvall, 1999). Also, ectopic grafts in the striatum may never be able to fully reverse all functional deficits because they cannot completely reconstruct normal nigrostriatal circuitry (Björklund *et al.*, 1994; Lindvall, 1999). Moreover, in Parkinson's disease some symptoms are probably caused by non-dopaminergic deficits and not amenable to recovery following dopamine neuron replacement (Lindvall, 1999). Nevertheless, current clinical trials are focused on improving functional outcome by optimizing the dopaminergic reinnervation of the caudate and putamen. Reproducible survival of dopaminergic grafts in the putamen has already been clearly demonstrated, and the magnitude of the survival in this structure broadly corresponds to the degree of recovery of motor function (Wenning *et al.*, 1997; Hagell *et al.*, 1999; Hauser *et al.*, 1999). However, it should be pointed out that the reinnervation of the putamen has been incomplete in the studies performed so far (Kordower *et al.*, 1995, 1996, 1998). Furthermore, no objective evidence of graft survival in the caudate has been obtained using PET, and it is therefore unclear whether tissue implanted into the caudate nucleus has contributed to the symptomatic relief after transplantation (Remy *et al.*, 1995; Wenning *et al.*, 1997; Hagell *et al.*, 1999).

In the present study, we have implanted human embryonic mesencephalic tissue bilaterally into both the caudate and putamen in five patients with Parkinson's disease (numbers 12–16 in the Lund series). The objectives have been two-fold: first, to explore the possibility that smaller amounts of tissue, compared with those used in previous clinical trials, can give rise to similar functional effects and dopaminergic graft survival if the transplant is exposed to the lazaroid, tirilazad mesylate; and secondly, to determine whether PET can provide evidence for significant survival of lazaroid-treated, dopaminergic grafts in the caudate nucleus and, if this is the case, to analyse whether caudate + putamen grafts give rise to a different pattern of motor recovery compared with putamen grafts alone.

Material and methods

Patients

Five patients were selected and followed according to the Core Assessment Program for Intracerebral Transplantations (CAPIT) (Langston *et al.*, 1992) either in Munich/Marburg (patients 12 and 13), Lund (patients 14 and 16) or London (patient 15). Patients' details are shown in Table 1. The patients' consent was obtained according to the Declaration of Helsinki and the procedures were approved by the local Ethical Committees in Lund (Research Ethics Committee, Lund University), London (The National Hospital for Neurology and Neurosurgery and the Institute of Neurology Joint Medical Ethics Committee, and Imperial College School of Medicine/Hammersmith, Queen Charlotte's & Chelsea and Acton Hospitals Research Ethics Committee) and Munich (Ethics Committee of the Medical Faculty, Ludwig-Maximilians-University of Munich).

Table 1 *Characteristics of patient group*

	Patient number				
	12	13	14	15	16
Age (years)[a]	53	41	49	54	68
Duration of disease/L-dopa treatment (years)[a]	15/15	13/13	11/8	12/8	12/11
No. of donors (left/right)	4/3	4/5	4/4	4/4	3/4
Follow-up (months)	24	24/18[b]	24	18	18
Hoehn & Yahr[a,c]	V	III-IV	III	III	III

[a]At the time of grafting; [b]for the first/second graft, respectively; [c]in practically defined 'off'.

Preparation of graft tissue

Apart from one change, i.e. the addition of the lazaroid tirilazad mesylate during each step of the procedure, the graft tissue was prepared according to the same protocol as in our previous series of patients (Wenning *et al.*, 1997; Hagell *et al.*, 1999). Briefly, dissociated ventral mesencephalic tissue from seven to nine aborted human embryos (aged 5–7 weeks post-conception; crown to rump length of 13–27 mm) was implanted in each patient (Table 1). The ventral mesencephalon was dissected from each embryo and cut into 6–10 pieces. These were stored for 2–5 h during the retrieval and dissection of donor tissue in a hibernation medium (Sauer and Brundin, 1991) supplemented with 3.0 μM tirilazad mesylate (Freedox®; Upjohn, Puurs, Belgium). Tissue pieces from multiple embryos were then incubated at 37°C for 20 min in Hanks' balanced salt solution (HBSS; Life Technologies, Täby, Sweden) with 0.1% trypsin (Worthington; Lakewood, NJ, USA) and 0.05% deoxyribonuclease (DNase; Sigma, Lund, Sweden) supplemented with 3.0 μM tirilazad mesylate. After incubation, the pieces were rinsed five times using HBSS containing 0.05% DNase and 3.0 μM tirilazad mesylate. Immediately before the first implantation, the pieces were mechanically dissociated using fire-polished Pasteur pipettes in HBSS with 0.05% DNase and 3.0 μM tirilazad mesylate.

Graft procedures

Implantation of embryonic mesencephalic tissue was performed in Lund using CT- and MRI-guided stereotaxic neurosurgery.

In all patients (Table 1), the grafts were placed bilaterally in the putamen and head of the caudate nucleus along five and two trajectories, respectively. The neurosurgical procedure is described in detail elsewhere (Lindvall *et al.*, 1989; Rehncrona, 1997). Patients 12 and 16 were grafted bilaterally in one session, whereas patients 14 and 15 were operated with an interval of 4 and 2 weeks, respectively, between the two sides. Patient 13 received his second graft (right striatum) 6 months after the first graft (left striatum).

Tirilazad mesylate was given intravenously to the patients four times a day (1.5 mg/kg) for 3 days, starting perioperatively at the time of the first implantation. All patients were immunosuppressed with cyclosporine, azathioprine and prednisolone according to a standard regimen (Lindvall *et al.*, 1989). Apart from patient 14 (azathioprine discontinued during the first month due to a liver reaction), this regimen was maintained for 12–24 months.

Pre- and postoperative follow-up

The patients were followed according to the CAPIT protocol (Langston *et al.*, 1992) from 6 months preoperatively to 18–24 months after surgery. Detailed descriptions of the clinical evaluation program are provided elsewhere (Lindvall *et al.*, 1989; Langston *et al.*, 1992). Medication was adjusted according to requirements.

The viability of the graft was assessed by measuring 6-L-[^{18}F]fluorodopa uptake using PET preoperatively and then postoperatively for 10–23 months. This was carried out at the MRC Cyclotron Unit at the Hammersmith Hospital, London, using a Siemens ECAT 953 PET scanner.

Details for the individual patients are summarized in Table 1.

Results

Patient and transplant details

The five patients included in this study had idiopathic Parkinson's disease (Gibb and Lees, 1988) with a mean duration of 12.6 years and had received L-dopa treatment for 11 years at the time of transplantation. They were in an advanced stage of the disorder (Hoehn and Yahr stage III–V in 'off'). In a direct comparison between the present patient group and a series of patients previously grafted in Lund, without lazaroid pretreatment of the implanted tissue (Wenning *et al.*, 1997; Hagell *et al.*, 1999), no differences in preoperative patient characteristics were evident (Table 2). Grafts were placed bilaterally in the caudate nucleus and putamen with tissue from three to five donors per side. The amount of donor tissue implanted in each putamen (obtained from a mean of 2.8 donors) was 42% less (Student's unpaired *t*-test, $P < 0.0001$; Table 2) than in our previous series of patients (mean: 4.8 donors; Wenning *et al.*, 1997; Hagell *et al.*, 1999) who received graft tissue that had not been subjected to lazaroid treatment. The patients were followed clinically and with [^{18}F]fluorodopa-PET for up to 18–24 months after surgery.

Changes in medication

In the first 18–24 months following surgery, all patients reduced their L-dopa dose on average by 54% compared with preoperatively (Table 3). Antiparkinsonian drugs were adjusted as follows.

Table 2 *Comparison between patient groups receiving grafts with and without lazaroid pretreatment*[a]

	This patient group[b]	Previous patient group[c]	*P*-value[d]
Age (years)	53 (41–68)	48.8 (43–53)	0.412
Duration of disease (years)	12.6 (11–15)	11.6 (7–16)	0.554
Duration of L-dopa treatment (years)	11 (8–15)	10.4 (7–15)	0.762
Hoehn and Yahr[e]	3.5 (3–5)	3.4 (2–4)	0.827[f]
Preoperative clinical scores:			
UPDRS motor score[e]	41.7 (23–67)	40.5 (30–57)	0.917[f]
Percent time in 'off'	29.9 (11.2–50.4)	38.0 (20.8–51.8)	0.469
Percent time in 'on' with dyskinesias	28.9 (3.9–50.4)	25.1 (0–45.8)	0.758
Time to perform 20 pronations/supinations[e]	32.2 (11.1–156.6)	41.5 (14.2–89.0)	0.578
Upper limb rigidity[e]	2 (1–4)	2.5 (1.7–3)	0.102[f]
Daily L-dopa dose (mg)	825.0 (225–1500)	700.0 (300–1000)	0.647
Duration of L-dopa induced 'on' (min)	115.1 (67–157)	112.4 (85–129)	0.902
Number of donors	2.8 (2.2–3.6)	4.8 (3.3–7.0)	<0.0001

[a]Preoperative group values. Data are presented as mean (range). [b]Grafts pretreated with tirilazad mesylate. [c]Grafts not pretreated with tirilazad mesylate (Wenning *et al.*, 1997; Hagell *et al.*, 1999). [d]Comparison between patient groups according to Student's unpaired *t*-test (except where indicated). [e]During practically defined 'off'. [f]Comparison between patient groups according to the Mann–Whitney *U*-test for non-parametric data.

In patient 12, there was no change in medication during the first 15 months postoperatively, when peak of dose dyskinesia necessitated a reduction in L-dopa medication. Ropinirole was added 20 months after surgery, with gradually increasing doses over the subsequent 7 months. By the end of the 24 month follow-up period, the patient was taking 6 mg of ropinirole per day.

In patient 13, pergolide (0.5 mg/day) and selegiline (10 mg/day), as well as L-dopa (225 mg/day) were withdrawn at 5–6 months after the second graft, and biperiden was reduced from 8 to 4 mg/day.

Patient 14 was on the same doses of selegiline (5 mg/day) and bromocriptine (7.5 mg/day) during the follow-up period, whereas L-dopa was reduced from 425 to 250 mg/day.

In patient 15, whose functional scores did not change (see below), pergolide (1.5 mg/day) and apomorphine self-injections (approximately 15 mg/day) were discontinued during the first postoperative year, and L-dopa was reduced from 900 to 400 mg daily, whereas amantadine (200 mg/day) was unchanged.

In patient 16, selegiline (10 mg/day) was withdrawn immediately after transplantation, and L-dopa was eventually reduced from 1075 to 725 mg/day.

Clinical status

The clinical data for the five patients, comparing the preoperative findings with those during the second postoperative year, are presented in Table 3. Virtually no change was observed postoperatively in one patient (patient 15; increase in his Unified Parkinson's Disease Rating Scale (UPDRS) motor examination score by 13%), but this patient had been able to markedly reduce his antiparkinsonian medication (see above). In contrast, the remaining four patients exhibited a markedly decreased UPDRS motor examination score in practically defined 'off' (Table 3; mean 48% reduction, range 37–58%). The percent time in 'off' phase decreased in four cases (not in patient 12). The postoperative changes of 'on' time with dyskinesias varied between the patients, with both increases (patient 13) and decreases (patient 15) being observed (Table 3). The time taken to perform motor tasks, e.g. pronations–supinations, in practically defined 'off', improved in all patients on the initially more severely affected side, and to a lesser degree on the other side also (Table 3). Rigidity decreased bilaterally in four of the patients, with patient 15 again exhibiting no major change. In patient 13, rigidity was moderate to severe preoperatively, and disappeared completely after transplantation (Table 3). No major alteration of the duration of the response to a single dose of L-dopa could be detected in any of the patients (Table 3). It should, however, be pointed out that no clear differences between 'on' and 'off' could be distinguished in patient 13 during the tests from 7 months and onwards after the second transplantation.

According to the UPDRS scores in practically defined 'off', swallowing, speech, gait, postural stability, posture and arising from sitting were only mildly to moderately affected preoperatively in four cases (data not shown). In the fifth case, swallowing, speech and posture were moderately to severely impaired before surgery. During the second postoperative year, we observed modest improvement of swallowing (one patient); speech (two patients); arising from sitting (four patients); posture (four patients); gait (one patient); and postural stability (two patients).

Graft viability

Regional [^{18}F]fluorodopa influx rate constant (K_i) values in the caudate nucleus and putamen before and after bilateral transplantation are shown for the individual patients in Fig. 1. At 10–23 months post-surgery, mean putaminal K_i had increased by 55% on the right side and 66% on the left side.

Table 3 *Clinical follow-up*

	Patient 12		Patient 13		Patient 14	
	Preop.[a]	Postop.[b]	Preop.	Postop.	Preop.	Postop.
UPDRS motor score in 'off'[c,d]	67 ± 58–69.5	42 ± 34.5–45.5	45 ± 43–48.75	21 ± 14.5–22.5[e]	38 ± 34.5–38.5	16 ± 9.5–18
Per cent time in 'off'[f]	13.1 ± 1.2	18.8 ± 0.5	11.2 ± 1.6	0 ± 0[e]	49.6 ± 0.9	34.9 ± 2.3
Per cent time in 'on' with dyskinesias[f]	19.7 ± 1.1	16.0 ± 0.4	3.9 ± 0.8	38.6 ± 3.3[e]	50.4 ± 0.9	35.5 ± 3.2
Time to perform 20 pronations/supinations (s), left[c,f]	156.6 ± 84.7	35.5 ± 18.9	19.1 ± 6.9	9.5 ± 0.8[e]	34.2 ± 4.1	17.5 ± 1.5
Time to perform 20 pronations/supinations (s), right[c,f]	32.2 ± 14.8	22.25 ± 7.0	13.7 ± 1.6	9.6 ± 0.8[e]	15.6 ± 1.4	13.5 ± 0.6
Arm rigidity score, left[c,d,g]	4 ± 2.75–4	1 ± 0–1.5	3 ± 2–3	0 ± 0–0.5[e]	2 ± 2–2.25	1 ± 0.5–1
Arm rigidity score, right[c,d,g]	3 ± 2.75–3	1 ± 1–2	2 ± 2–3	0 ± 0–1[e]	1.5 ± 1–1.5	0 ± 0–0
Daily L-dopa dose (mg)[h]	1500	900	225	0[f]	425	250
Duration of L-dopa induced 'on' (min)[i,j]	67 (45–140)	103 (90–150)	157 (105–215)	k	68.0 (60–75)	108 (50–155)

	Patient 15		Patient 16	
	Preop.	Postop.	Preop.	Postop.
UPDRS motor score in 'off'[c,d]	23 ± 17.75–26.75	26 ± 26–29.5	35.5 ± 34.25–36.5	20.5 ± 18.5–23.5
Per cent time in 'off'[f]	25[l]	11.9 ± 3.5	50.4 ± 1.7	20[l]
Per cent time in 'on' with dyskinesias[f]	50[l]	3.3 ± 1.6	20.7 ± 1.4	15[l]
Time to perform 20 pronations/supinations (s), left[c,f]	11.1 ± 1.0	9.1 ± 0.4	14.4 ± 0.5	9.9 ± 0.1
Time to perform 20 pronations/supinations (s), right[c,f]	12.5 ± 1.2	10.0 ± 0.4	12.9 ± 0.5	10.0 ± 0.2
Arm rigidity score, left[c,d,g]	1 ± 1–1	1 ± 1–1.25	1 ± 1–1	0 ± 0–0.75
Arm rigidity score, right[c,d,g]	1 ± 1–1	1 ± 1–1.25	1 ± 1–1	0.5 ± 0–1
Daily L-dopa dose (mg)[h]	900	400	1075	725
Duration of L-dopa induced 'on' (min)[i,j]	153.3 (115–220)	95 (60–140)	130.4 (120–140)	119.25 (105–132)

[a]Assessments from 6 months prior to transplantation. [b]Assessments from the second year after transplantation. [c]During practically defined 'off'. [d]Median ± 25th percentile. [e]Assessments from 13–24 and 7–18 months after first and second transplantation, respectively. [f]Mean ± 95% confidence interval. [g]According to the UPDRS. [h]During the 6 months prior to transplantation and at the end of the follow-up period. [i]Duration of the 'on'–phase after a single dose of L-dopa. [j]Mean (range). [k]Not possible to distinguish 'on' from 'off' phase during single-dose L-dopa tests. [l]Regular autoscoring not available. Data based on historical information from the patient. UPDRS = Unified Parkinson's Disease Rating Scale; L-dopa = levodopa + a peripheral decarboxylase inhibitor.

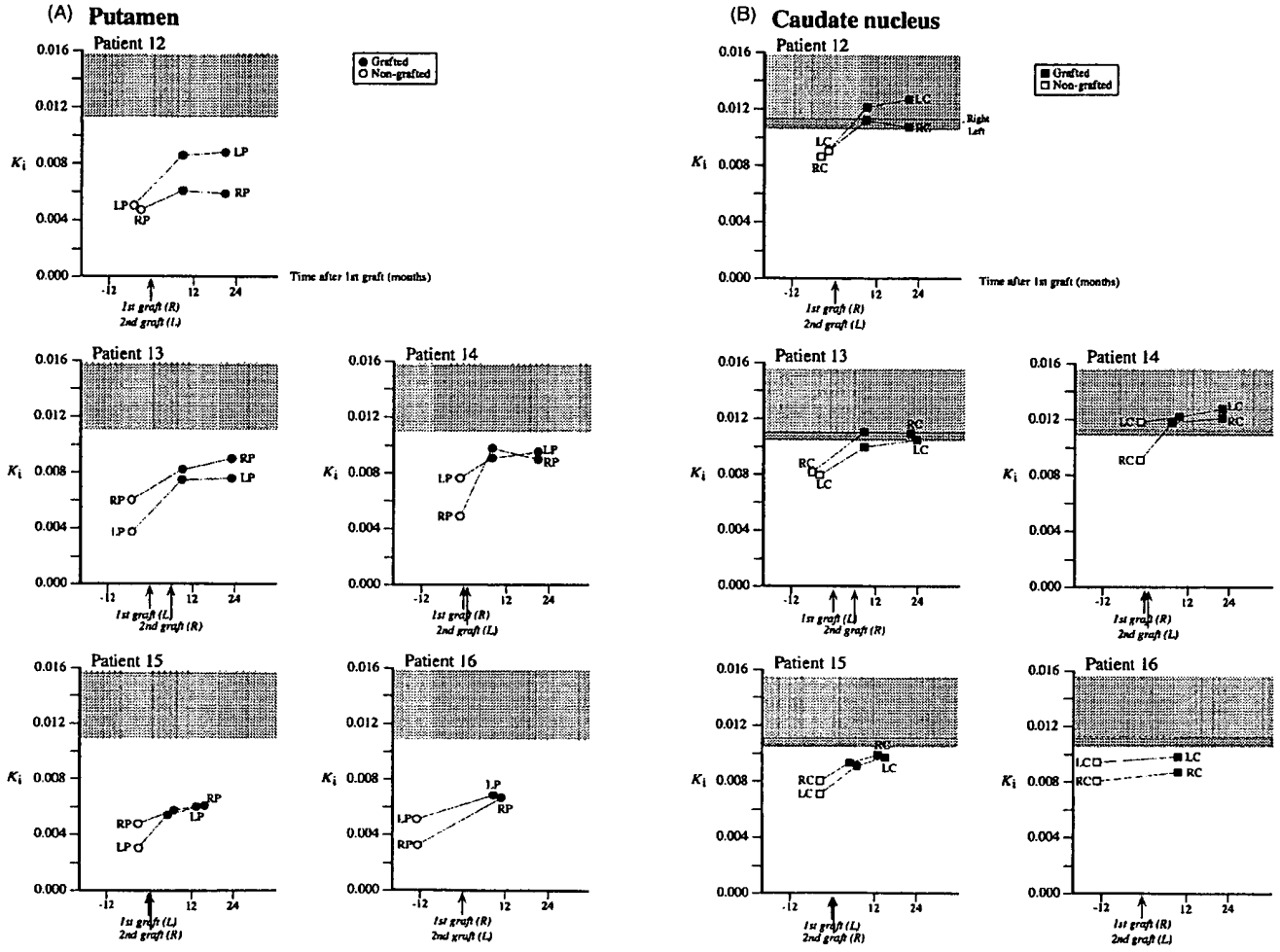

Fig. 1 Regional 6-L-[^{18}F]fluorodopa influx rate constant (K_i) after bilateral transplantation of embryonic mesencephalic tissue to the putamen (A; filled circles = grafted; open circles = non-grafted) and caudate nucleus (B; filled squares = grafted; open squares = non-grafted) in patients 12–16. Tirilazad mesylate was administered to the graft tissue prior to implantation and given intravenously to the patients for 3 days after transplantation. Comparative data are given for a group of 10 healthy volunteers. The shaded areas show the normal mean minus 2 SD. The numbers on the *x*-axes refer to month before (minus) and after the first graft. RP = right putamen; LP = left putamen; RC = right caudate nucleus; LC = left caudate nucleus.

The increases ranged from 25 to 26% in the left putamen in patient 14 and right putamen in patients 12 and 15 to 99–101% in the left putamen in patient 13 and 15 and right putamen in patient 16. Compared with the mean obtained from a group of normal subjects, the putaminal K_i influx rate constant value for the current patient group reached 47% on the right and 49% on the left side, and failed to reach the normal range (i.e. within 2 SD of the normal mean) at 10–23 months post-surgery. The change of K_i in the caudate nucleus was less pronounced than in the putamen, the mean increase being 24% on both sides. Postoperatively, the K_i value in the caudate nucleus was 67% of the normal mean. Interestingly, significant increases (20% or more) of [^{18}F]fluorodopa uptake were detected uni- or bilaterally in the caudate nucleus in four patients (Fig. 1).

Side-effects

Tirilazad mesylate gave rise to peripheral thrombophlebitis in all patients. This was mild and successfully treated with topical heparin except in patient 15, who developed severe thrombophlebitis after the first transplantation. Patient 12 exhibited mild nocturnal confusion during the first 3 days following bilateral surgery and experienced periods of mild depression during the follow-up period. Patient 15 had a slight change of personality and slight apathy during the first few days after his second graft. Following bilateral transplantation in one session, patient 16 (at 68 years, the oldest subject by 14 years) exhibited confusion lasting for 1 week. Subsequently he developed depression, which necessitated treatment. His mild preoperative cognitive impairment deteriorated slightly postoperatively.

Discussion

The present data demonstrate that transplantation of human embryonic mesencephalic tissue bilaterally into the caudate nucleus and putamen can lead to significant symptomatic relief in patients with Parkinson's disease. During the second postoperative year, all five patients had decreased their daily L-dopa dose, and four of them exhibited a marked reduction of UPDRS motor score in 'off' phase, with one patient even lacking distinctive 'off' periods. Changes in antiparkinsonian medications were performed as required by the patients' postoperative clinical development. Apart from in patient 12, in whom ropinirole (6 mg/day) was added during the second postoperative year, these changes comprised drug withdrawals or decreased dosages. The addition of ropinirole in patient 12 was paralleled by a 40% reduction in daily L-dopa dose. All patients showed increased [^{18}F]fluorodopa uptake bilaterally in the putamen, and four of them also in the caudate nucleus. The two cases with the most marked clinical improvement (patients 13 and 14) reached relatively high putaminal [^{18}F]fluorodopa uptake (close to normal mean minus 2 SD) on both sides after grafting. In contrast, the putaminal [^{18}F]fluorodopa uptake in the other patients was clearly lower, either uni- (patient 12) or bilaterally (patients 15 and 16). The histopathological studies of Kordower and colleagues have confirmed that increased [^{18}F]fluorodopa uptake on PET reflects the survival of dopaminergic grafts reinnervating the patient's striatum (Kordower *et al.*, 1995, 1996, 1998). Therefore, it is probable that the improvements in our patients were due to survival of dopaminergic neurons in the grafts.

Animal experiments indicate that the massive death of grafted embryonic dopaminergic neurons occurs during the first week after transplantation (Duan *et al.*, 1995; Barker *et al.*, 1996; Zawada *et al.*, 1998; Emgård *et al.*, 1999; Schierle *et al.*, 1999). A major portion of these cells probably dies through an apoptotic mechanism (Schierle *et al.*, 1999), although necrosis is also likely to underlie some neuronal loss. In animals, the survival of grafted dopaminergic neurons can be improved by the administration of growth factors (Mayer *et al.*, 1993; Takayama *et al.*, 1995; Rosenblad *et al.*, 1996; Sinclair *et al.*, 1996; Zeng *et al.*, 1996; Sautter *et al.*, 1998; Sullivan *et al.*, 1998; Yurek *et al.*, 1998; Zawada *et al.*, 1998; Wilby *et al.*, 1999) and compounds which reduce oxidative stress (Nakao *et al.*, 1994; Grasbon-Frodl *et al.*, 1996; Björklund *et al.*, 1997; Karlsson *et al.*, 1999; Hansson *et al.*, 2000) or inhibit caspases (Schierle *et al.*, 1999; Hansson *et al.*, 2000). The present study is the first attempt to explore whether any of these strategies can also increase graft survival in patients. Here we have used the lazaroid tirilazad mesylate, which inhibits lipid peroxidation (Braughler *et al.*, 1989). Similar to two other related compounds (Frodl *et al.*, 1994; Nakao *et al.*, 1994; Grasbon-Frodl *et al.*, 1996; Karlsson *et al.*, 1999), tirilazad mesylate promotes the survival of embryonic rat and human dopamine neurons (Björklund *et al.*, 1997; Othberg *et al.*, 1997; Hansson *et al.*, 2000). Administration of tirilazad mesylate supports a two-fold increase of the number of surviving cultured rat mesencephalic dopamine neurons (Othberg *et al.*, 1997), and similar increases in the survival of rat dopamine neurons grafted to the striatum (Hansson *et al.*, 2000) or anterior chamber of the eye (Björklund *et al.*, 1997). Furthermore, tirilazad mesylate significantly prolongs the time during which both rat and human embryonic mesencephalic cell suspensions display high viability when stored at room temperature (Othberg *et al.*, 1997).

In the patients reported here, lower amounts of embryonic mesencephalic tissue were implanted in the striatum, compared with our previous cases with bilateral grafts (Table 2). In patients 3 and 7–10, who were also grafted bilaterally and whose preoperative characteristics and symptomatology were comparable to the present patients (Table 2; Wenning *et al.*, 1997; Hagell *et al.*, 1999), we implanted a mean of 4.8 ventral mesencephali in each putamen. In addition, 2.2 ventral mesencephali were implanted into each caudate nucleus bilaterally in one patient, and unilaterally in another. The current patients only received a mean of 2.8 and 1.1 mesencephali in the putamen and caudate nucleus, respectively. Thus, the amount of implanted tissue in the lazaroid-group was 42% less in the putamen and 50% less in the caudate nucleus compared with patients 3 and 7–10. Nonetheless, graft survival (as measured by PET) and the magnitude of the symptomatic relief seem to be similar in the two groups. In PET scans performed between 6 and 12 months after transplantation, the mean increase of [^{18}F]fluorodopa uptake compared with preoperatively in the grafted putamina, was identical in patients 3 and 7–10 ($+60 \pm 27\%$; $n = 8$, with each putamen receiving grafts treated as an individual case) and patients 12–16 ($+60 \pm 35\%$; $n = 9$). Similarly, both groups showed the same postoperative elevation of putaminal [^{18}F]fluorodopa uptake in scans carried out 13 to 24 months after transplantation ($+60 \pm 46\%$; $n = 6$; and $+60 \pm 33\%$; $n = 8$, respectively). A recent experimental study is particularly relevant to these findings (Sullivan *et al.*, 1998). Using the PET technique in rats, it revealed a correlation between a growth factor treatment-induced increase in survival of grafted nigral dopamine neurons and striatal binding of [^{11}C]RTI-121, a marker for dopaminergic nerve terminals (Sullivan *et al.*, 1998).

The clinical improvements during the second year after bilateral transplantation in patients 3, 7, 9 and 10 were similar to those in patients 12–16. Patient 8 was excluded from this comparison because he has atypical parkinsonism, possibly multiple system atrophy (Wenning *et al.*, 1997; Hagell *et al.*, 1999). In patients 3, 7, 9 and 10, the mean UPDRS motor examination score in practically defined 'off' decreased from 43 to 30, i.e. by 30%. Similarly, the score in patients 12–16 decreased by 40%, from 42 to 25. One patient in each group (number 7 and 13, respectively) was able stop L-dopa treatment completely. Taken together, these findings are in agreement with observations in experimental animals (Nakao *et al.*, 1994; Grasbon-Frodl *et al.*, 1996; Björklund *et al.*,

1997; Karlsson *et al.*, 1999; Hansson *et al.*, 2000) and provide tentative evidence that lazaroid treatment improves survival of grafted human embryonic dopamine neurons also in patients.

In all cases, except patient 16, we observed a significant increase of [^{18}F]fluorodopa uptake (by 23–41%) uni- or bilaterally in the caudate nucleus. Previous studies have failed to provide any PET data that clearly indicate survival and growth of dopaminergic grafts in this structure (Remy *et al.*, 1995; Wenning *et al.*, 1997; Hagell *et al.*, 1999). This has been attributed to the amounts of implanted tissue being too small; the possibility that conditions for graft survival are less favourable in the caudate nucleus, and difficulties with the PET technique in this region (Wenning *et al.*, 1997). At autopsy, Kordower and colleagues observed growth of dopaminergic fibres into the caudate nucleus from implants placed in the putamen (Kordower *et al.*, 1996), but there were no grafts placed directly into the caudate nucleus in that patient. We provide here the first evidence using PET that dopaminergic grafts can survive in the caudate nucleus. It should be emphasized that the amount of embryonic mesencephalic tissue implanted in the present patients (0.9–1.4 mesencephali) was clearly lower than that grafted in our previous cases (1.7–2.0 mesencephali). Therefore, the significant increase of [^{18}F]fluorodopa uptake in the caudate nucleus observed here may be interpreted as further support that tirilazad mesylate increases the survival of grafted dopaminergic neurons. It may, however, also be due to a PET scanner with higher resolution being used in the present patients than in cases 3 and 7–10.

The PET scans revealed a difference between the side of the brain operated first and the side operated in the second session, both with respect to the absolute and relative increase of putaminal [^{18}F]fluorodopa uptake after grafting. The relative increase, compared with preoperatively, ranged between 74 and 101% (mean 91%) in the putamen with the most pronounced postoperative change, and between 25 and 50% (mean 31%) on the contralateral side. These findings suggest that there is a corresponding side difference in the number of surviving grafted dopamine neurons. This side difference was not correlated with the amount of mesencephalic tissue implanted in each putamen. In fact, four patients received more mesencephalic donor tissue on the side with less change of [^{18}F]fluorodopa uptake, or equal amounts on both sides. Only one patient (patient 12) had been grafted with more tissue on the side with the most marked increase of [^{18}F]fluorodopa uptake. In patients 13–16, the putamen with the most pronounced change after grafting had the lowest preoperative [^{18}F]fluorodopa uptake. This suggests that there is better graft survival when the degeneration of the intrinsic dopamine system is more advanced, although earlier comparisons of nigral graft size in rats with an intact or lesioned nigrostriatal pathway do not support this idea (Doucet *et al.*, 1990). It is possible that bilateral transplantation surgery performed at the same or two closely spaced surgical sessions compromises the survival of one of the grafts due to an unknown mechanism, e.g. an inflammatory response that affects the basal ganglia bilaterally. In the present study, the first grafted putamen exhibited the most marked increase in [^{18}F]fluorodopa uptake (except in patient 12, who was grafted bilaterally in the same session). Previously, we made the same observations in two patients with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced parkinsonism who were grafted bilaterally with a 2 week interval (Widner *et al.*, 1992). They displayed a difference in both absolute and relative change of fluorodopa uptake, compared with preoperatively, between the striatum receiving the first and second graft (mean 81 and 54% increase, respectively). This side difference was not apparent in our previous series of idiopathic Parkinson's disease patients (numbers 3, 7, 9 and 10), who were grafted sequentially with time intervals of between 10 and 56 months (Wenning *et al.*, 1997; Hagell *et al.*, 1999). Although these observations await explanation, they seem to suggest that bilateral transplantation within a short time interval may be less favourable for survival of the second graft. Because patient 4 of our series has revealed that striatal fluorodopa uptake can continue to increase up to at least 3 years after transplantation surgery (Piccini *et al.*, 1999), the observed side difference in the present series of patients needs to be followed up over time.

The patients described here are the first with idiopathic Parkinson's disease who have been grafted bilaterally in the caudate nucleus and putamen. In four cases (all except patient 16), PET indicated survival of the dopaminergic grafts in both the putamen and caudate nucleus. However, data from the neurological evaluation do not provide any evidence for a different pattern of recovery of motor function in these patients. Similar to patients with surviving grafts only in the putamen (Defer *et al.*, 1996; Wenning *et al.*, 1997; Hagell *et al.*, 1999; Hauser *et al.*, 1999), the patients of the current study exhibited moderate to major reductions of hypokinesia and rigidity in 'off' and of the time spent in the 'off' phase. Furthermore, both groups of patients displayed only modest, inconsistent improvements of gait, arising, posture, postural stability, swallowing and speech. The effect on dyskinesias varied between the patients, with some showing increases and others clear reductions. However, no tests specifically addressing caudate-prefrontal functions (e.g. fluency and problem solving) were performed and therefore it cannot be exluded that such functions were influenced by the surviving grafts in the caudate nucleus.

Two patients who were transplanted bilaterally in the same surgical session developed confusion postoperatively, lasting up to 1 week. One patient exhibited a change of personality during the first days after the second transplantation, which was performed 2 weeks after the first one. It is conceivable that these transient cognitive symptoms primarily reflect the surgical interventions in the caudate nucleus, which is considered to play a more prominent role in cognition than the putamen (Bhatia and Marsden, 1994; Holthoff-Detto *et al.*, 1997), and that the risks for adverse reactions are higher when bilateral implants are performed in the same session.

Even though the present study provides preliminary evidence that treatment with tirilazad mesylate may increase the yield of surviving dopamine neurons after grafting, relatively large amounts of human embryonic donor tissue are still needed for significant therapeutic effects to develop. If both sides of the brain are considered, each patient studied here received tissue from a total of between five and seven donors just in the putamen. This resulted in beneficial effects on motor function comparable to those observed in patients implanted with tissue, not treated with tirilazad mesylate, from between 9 and 11 donors (Wenning *et al.*, 1997; Hagell *et al.*, 1999). [^{18}F]Fluorodopa uptake increased significantly after transplantation, but did not reach normal levels, indicating that the graft-derived dopaminergic reinnervation was not complete. It is probable that a more complete reinnervation of the striatum, which would most likely increase the symptomatic relief, will require a higher number of surviving dopamine neurons, and maybe also stimulation of their axonal growth capacity. Therefore, in order to be able to graft large numbers of patients, strategies need to be developed which increase the yield of surviving dopamine neurons from each human embryonic donor at least 5- to 10-fold compared with untreated tissue. This may be possible to achieve in the future by combining methods to expand dopamine precursor cells (Studer *et al.*, 1998) with different approaches to limit neuronal death after transplantation (Brundin *et al.*, 2000), e.g. administration of neurotrophic factors (Mayer *et al.*, 1993, Takayama *et al.*, 1995; Rosenblad *et al.*, 1996; Sinclair *et al.*, 1996; Zeng *et al.*, 1996; Sautter *et al.*, 1998; Sullivan *et al.*, 1998; Yurek *et al.*, 1998; Zawada *et al.*, 1998; Wilby *et al.*, 1999), antioxidants (Nakao *et al.*, 1994; Grasbon-Frodl *et al.*, 1996; Björklund *et al.*, 1997; Karlsson *et al.*, 1999; Hansson *et al.*, 2000) and caspase inhibitors (Schierle *et al.*, 1999; Hansson *et al.*, 2000).

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